From the marrow to the blood: Optimising the diagnosis of iron deficiency in the setting of inflammation

By Dr David Richardson

RCHDAV012

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Department of Pathology, The University of Cape Town

Primary Supervisor:

Associate Prof. Jessica Opie

Division of Haematology, Department of Pathology, Faculty of Health Sciences, University of Cape Town and National Health Laboratory Service and Groote Schuur Hospital, Cape Town, South Africa

Co-Supervisor:

Prof. Vernon Louw

Chair and Head of Division of Clinical Haematology

Department of Medicine, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

Declaration Page

I, David Richardson, declare that this research reported is based on independent work that I have performed under supervision for the completion of my Masters of Medicine minor dissertation in Haematology, in the Department of Pathology.

Neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university.

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Student number: RCHDAV012

Signature: Signed by candidate

Date: 17 April 2024

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Acknowledgements, format, and contributions

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Abbreviations

AUC^{ROC}	Area under the receiver operating curve
CI	Confidence interval
CRP	C-reactive protein
EDTA	Ethylenediamine tetra-acetic acid
HIV	Human immunodeficiency virus
ID	Iron deficiency
MCV	Mean cell volume
MCH	Mean cell haemoglobin
OR	Odds ratio
RDW	Red cell distribution width
ROC	Receiver operating characteristic
RET-H*e*	Reticulocyte haemoglobin equivalent
SF	Serum ferritin
sTfR	Soluble transferrin receptor
TF	Transferrin
TSAT	Transferrin saturation

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Abstract

Aims: Iron deficiency (ID) is a common condition with readily available treatment but can be challenging to diagnose. Traditional biomarkers of ID are acute phase reactants, which complicates diagnosis in patients with co-existent inflammation. This study aimed to establish optimal biomarker diagnostic thresholds for ID diagnosis using bone marrow (BM) iron stores as the gold standard and the C-reactive protein (CRP) as an inflammatory marker.

Methods: A cross-sectional study was carried out in the haematology department of a tertiary academic hospital. Patients undergoing BM biopsies for any reason were recruited for inclusion. Retrospective case finding was used to enrich the data for cases with confirmed BM ID. Laboratory markers including red cell indices, reticulocyte haemoglobin and iron studies were evaluated to establish optimal cut-offs for ID diagnosis. A CRP of >5 mg/L was used as a marker of inflammation.

Results: The study included 139 patients. Forty-two patients had BM ID with a median serum ferritin (SF) of 48.5 µg/L. 96/134 (72%) had inflammation with a CRP > 5 mg/L. A SF of < 80 µg/L had optimal sensitivity (69%) and specificity (94%) for ID diagnosis in the whole group (OR 23.5; CI 4.3-129). In patients without inflammation, a SF 80 cut-off had high sensitivity (93%) and specificity (96%). A SF < 200 µg/L indicated ID in those with inflammation (sensitivity 78%, specificity 74%). A transferrin saturation of <13% in those with inflammation increased the diagnostic specificity (92%). The reticulocyte haemoglobin was unhelpful in diagnosing ID in this setting.

Conclusions: In this hospital population, SF was the best parameter to diagnose ID, even in the presence of inflammation, albeit at a higher cut-off level. The CRP was useful to identify populations in whom a higher SF threshold could be used together with the transferrin saturation to accurately diagnose ID.

Publication Ready Format

Title:

From the marrow to the blood: Optimising diagnosis of iron deficiency in the setting of inflammation

Authors:

David Richardson [1], Karryn Brown[2], Jody Rusch[3], Estelle Verburgh[2], Vernon Louw[2], Jessica Opie[1]

Affiliations:

1. Division of Haematology, Department of Pathology, Faculty of Health Science, University of Cape Town and National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa
2. Division of Clinical Haematology, Department of Medicine, University of Cape Town, and Groote Schuur Hospital, Cape Town, South Africa
3. Division of Chemistry, Department of Pathology, Faculty of Health Science, University of Cape Town and National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa.

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Anaemia, Iron deficiency, Prussian blue reaction, Bone marrow examination, Ferritin, Transferrin, Reticulocytes, C-reactive protein, HIV, Inflammation

Corresponding author's contact details:

David Richardson

C20 Laboratory, National Health Laboratory Service, Groote Schuur Hospital, University of Cape Town Department of Pathology, Observatory, 7925, Cape Town, South Africa

Email: david.richardson@nhls.ac.za Tel: +27 (0)84 533 1278

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Diagnosing iron deficiency in the setting of inflammation

Abstract

Iron deficiency (ID) is a common condition with readily available treatment but can be challenging to diagnose. Traditional biomarkers of ID are acute phase reactants, which complicates diagnosis in patients with co-existent inflammation. This study aimed to establish optimal biomarker diagnostic thresholds for ID diagnosis using bone marrow (BM) iron stores as the gold standard and the C-reactive protein (CRP) as an inflammatory marker.

A cross-sectional study was carried out in the haematology department of a tertiary academic hospital. Patients undergoing BM biopsies for any reason were recruited for inclusion. Retrospective case finding was used to enrich the data for cases with confirmed BM ID. Laboratory markers including red cell indices, reticulocyte haemoglobin and iron studies were evaluated to establish optimal cut-offs for ID diagnosis. A CRP of >5 mg/L was used as a marker of inflammation.

The study included 139 patients. Forty-two patients had BM ID with a median serum ferritin (SF) of 48.5 µg/L. 96/134 (72%) had inflammation with a CRP > 5 mg/L. A SF of < 80 µg/L had optimal sensitivity (69%) and specificity (94%) for ID diagnosis in the whole group (OR 23.5; CI 4.3-129). In patients without inflammation, a SF 80 cut-off had high sensitivity (93%) and specificity (96%). A SF < 200 µg/L indicated ID in those with inflammation (sensitivity 78%, specificity 74%). A transferrin saturation of <13% in those with inflammation increased the diagnostic specificity (92%). The reticulocyte haemoglobin was unhelpful in diagnosing ID in this setting.

In this hospital population, SF was the best parameter to diagnose ID, even in the presence of inflammation, albeit at a higher cut-off level. The CRP was useful to identify populations in whom a higher SF threshold could be used together with the transferrin saturation to accurately diagnose ID.

From the marrow to the blood: Optimising diagnosis of iron deficiency in the setting of inflammation

1. Introduction

Iron deficiency (ID) is a common medical condition with significant clinical sequelae. [1–4] Timely and accurate diagnosis of ID with or without anaemia should lead to prompt initiation of treatment, and appropriate investigations for the underlying cause. [3,5–8] Since both treatment and further investigations may be costly or have negative sequelae, accurate diagnosis is essential. [6,9] A bone marrow (BM) aspirate stained for iron stores, while considered the gold standard, is invasive, uncomfortable, costly, and labour-intensive. [3,10] Because of these limitations, the laboratory diagnosis of ID is typically based on peripheral blood biomarkers such as red cell indices and iron studies. However, interpretation of these biomarkers is frequently complicated by a co-existing inflammatory state which may be related to a patient's primary pathology or comorbidities. [1,10–16]

In the absence of inflammation, the serum ferritin (SF) correlates highly with cellular ferritin and is the best single biomarker for ID diagnosis with a high sensitivity and specificity. [17–20] However, as a positive acute phase reactant, SF increases in acute and chronic inflammatory states, and certain malignancies. Therefore, in patients with inflammation, using SF cut-offs to diagnose ID that are based on references from healthy populations (15 to 30 µg/L), results in under-diagnosis. [10,19–22] One approach to compensate for the effect of inflammation is to use increased SF cut-offs for ID diagnosis. Current disease-specific SF cut-offs include <300 µg/L in cardiac failure and inflammatory bowel disease, and <500 µg/L in chronic kidney disease. [13,23,24] Inflammatory markers, such as the C-reactive protein (CRP), have been used to select patients in whom increased SF cut-offs should be used to diagnose ID. [25–27]

A second approach to improving the diagnosis of ID is to use other biomarkers in conjunction with SF. Red cell indices such as a reduced mean cell volume (MCV) and mean cell haemoglobin (MCH) have a strong correlation with ID anaemia but develop gradually and may be masked with certain comorbidities, medications, and co-existing haematinic deficiencies. [3,6,18,19] The reticulocyte haemoglobin equivalent (RET-H*e*), available on Sysmex instruments, is useful in evaluating early ID and predicting and monitoring the response to iron therapy. [3,28,29] The RET-H*e* has been evaluated to discriminate anaemia of inflammation from ID, but there is poor consensus regarding optimal cut-offs and RET-H*e*'s role in diagnostic algorithms. [30] Similarly, reticulocyte haemoglobin content (CHr) and the percentage hypochromic red cells (%Hypo), available on Advia instruments, have shown benefit in detection of early iron deficiency, even in the presence of end-stage kidney disease. [29,30] Transferrin saturation (TSAT), calculated from serum iron and transferrin (Tf), is used with SF to diagnose concurrent ID and anaemia of inflammation, however, the recommended cut-offs for both parameters

vary in the literature. [3,4,17] The TSAT has limitations due to Tf being a negative acute phase response protein and serum iron being subject to diurnal variation and dietary intake.[10,17,19,21] The serum soluble transferrin receptor (sTfR) is considered a more reliable biomarker for ID in the setting of inflammation. However, sTfR has limited availability, poor assay standardisation, and the reference intervals are poorly defined in specific populations, including people living with HIV. [3,31,32] Furthermore, the use of ratios and combinations of various biomarkers, such as sTfR/SF, sTfR/log (SF) or Tf/log (SF) are suggested to be more accurate than using the values alone. However, the diagnosis of ID may be obscured by increased SF and reduced transferrin in inflammatory states. [10,28,33]

The plethora of diagnostic approaches indicates the lack of an ideal test for ID. Although some studies show that using higher SF cut-offs for patients with elevated CRPs improves the diagnosis of ID, this has not been widely accepted in current guidelines due to several limitations in the literature. [4,26] These limitations include a lack of BM data, the exclusion of patients with elevated inflammatory markers and variations in cut-offs used for SF, TSAT and RET-H*e* for ID diagnosis. [25–27] This study aimed to describe the relationship between BM ID, peripheral blood biomarkers and CRP in an effort to optimise the diagnosis of ID.

2. Material and methods

2.1 Study Design

This was a cross-sectional, descriptive study conducted in the Division of Clinical Haematology at Groote Schuur Hospital, a 975-bed tertiary academic hospital in Cape Town, South Africa. The study was approved by the University of Cape Town Human Research Ethics Committee (HREC 302/2021) and conducted according to the ethical principles as contained in the Declaration of Helsinki.

2.2 Study Population

A prospective study design was initially employed with consecutive sampling of all adult patients (≥18 years old) referred to Clinical Haematology for a BM biopsy who were not receiving chemotherapy, iron therapy or had received a recent blood transfusion. Between August 2021 and March 2022 informed consent was obtained from all eligible patients. Patients were excluded if iron studies were not performed or if the BM aspirate slides were inadequate for the assessment of iron status. Due to the low yield of ID participants and to pragmatically increase the total number of ID patients for statistical analysis, retrospective case-finding was employed in a second phase. In the retrospective analysis, the BM data base (HREC R018/2017) was reviewed and only patients with reduced BM iron stores and complete iron studies between March 2022 and March 2023 were considered for inclusion. All patients with ID who met eligibility criteria were considered for enrolment in the study and informed consent was obtained prior to data capture (Figure 1).

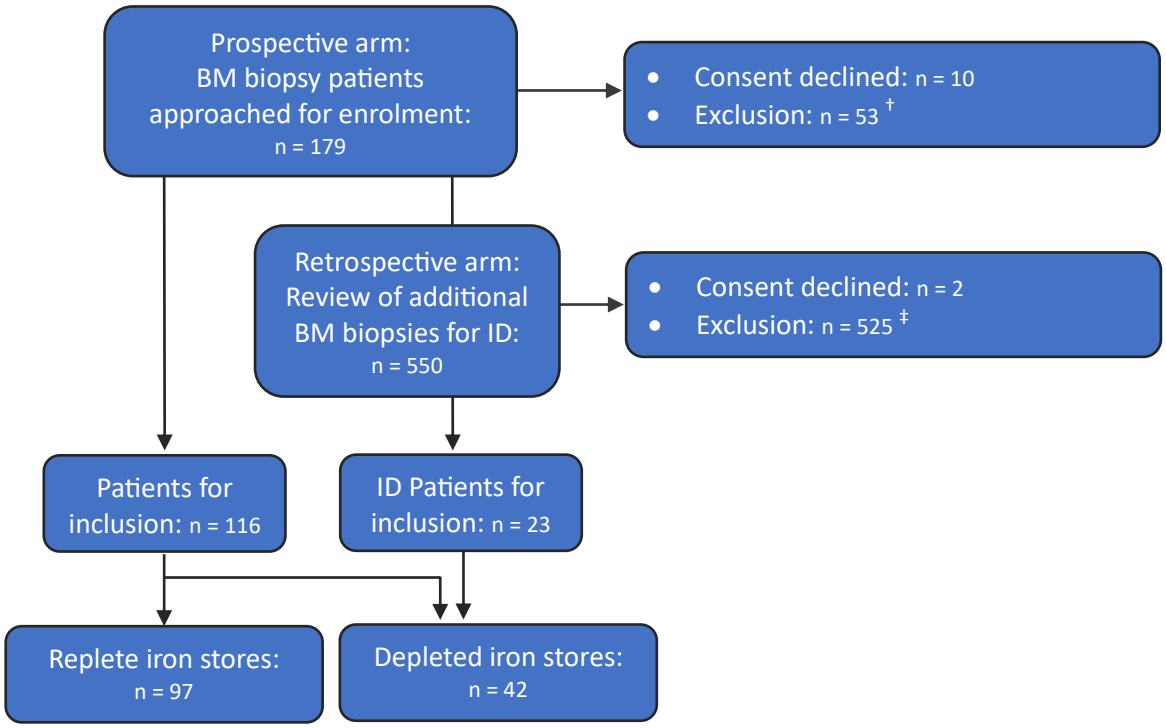

Figure 1: Study design and numbers:

† Iron stain unsuitable for grading (n=37); Incomplete data (n=10); Chemotherapy, iron therapy or recent blood transfusion identified on folder review (n=6)

‡ Age < 18 years (n=1); Replete iron stores or insufficient particles for grading (n=510); Incomplete data (n=9); Chemotherapy, iron therapy or recent blood transfusion identified on folder review (n=5)

BM – Bone marrow; ID – Iron deficiency

2.3 Laboratory Tests

All laboratory tests were performed in the National Health Laboratory Service (NHLS) clinical pathology laboratory in Groote Schuur Hospital, accredited by the South African National Accreditation System, which adheres to international quality standards (ISO 15189). CRP and iron studies were performed as part of routine patient care. These tests were typically performed on the day of the BM biopsy, but were included if collected within 5 days of the BM biopsy.

2.3.1 Bone Marrow Assessment of Iron Status

Bone marrow aspirate slides were stained with Perls' Prussian blue stain and a minimum of seven particles were reviewed for assessment of iron stores by a qualified Haematology Pathologist. [34] The iron content of the particles was graded between 0-6. [34,35] Patients with grade 0 (absent) or grade 1 (markedly reduced) BM iron stores were classified as iron deficient (Figure 2) in keeping with local standard operating procedures and similar publications. [36,37] A minimum of 100 erythroblasts were assessed for siderotic granules with <10% sideroblasts considered reduced. [35,38]

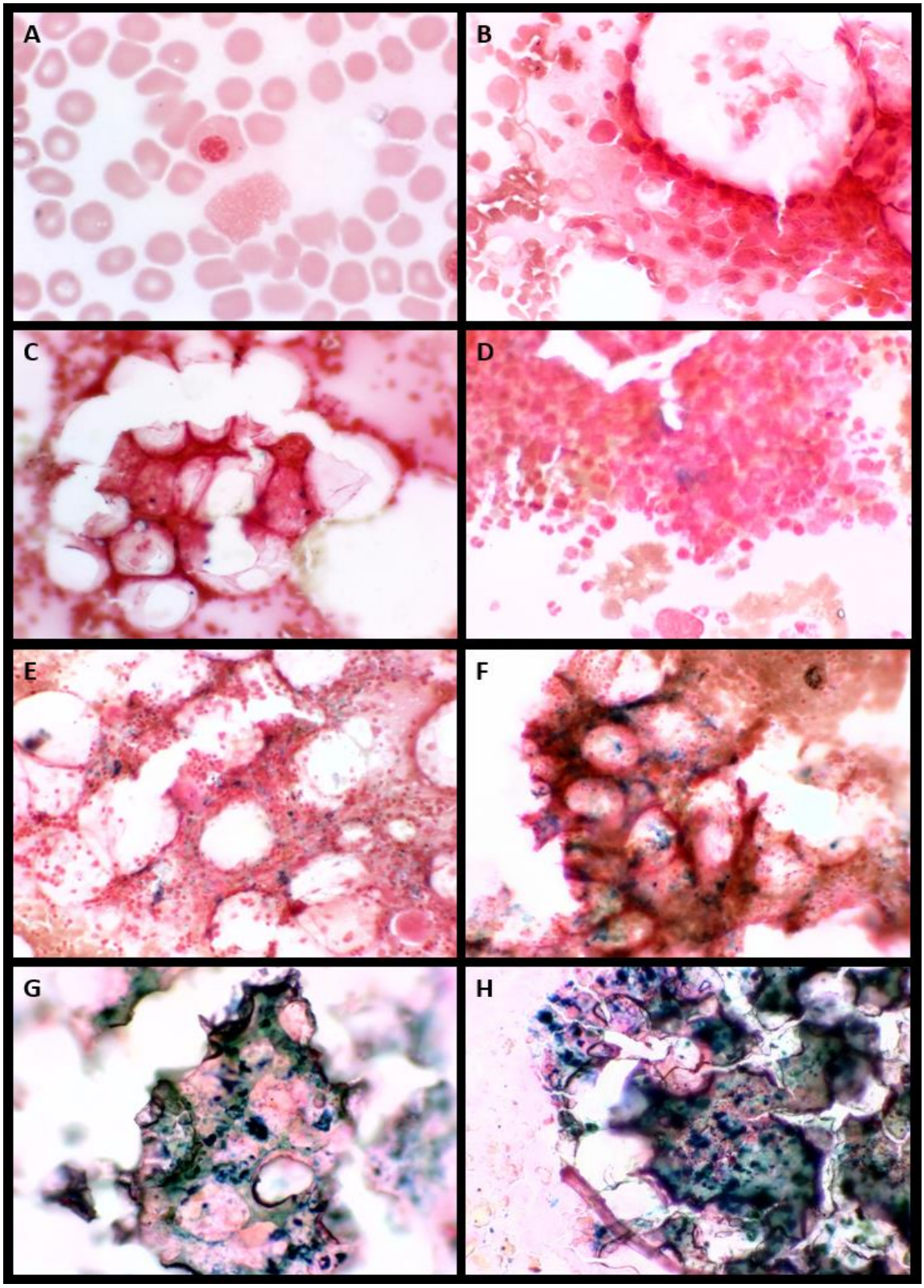

Figure 2: Grading of iron stains:

(A) A normal sideroblast with a single, small siderotic granule; (B*) No stainable iron visible under 50X high power – Grade 0 (iron deficient); (C) Small particles visible under 50X high power – grade 1 (iron deficient); (D) Sparse, small particles visible under low power – grade 2 (iron replete); (E) Numerous small particles – grade 3 (iron replete); (F) Larger particles with a tendency to aggregate into clumps – Grade 4 (iron overload); (G) Dense large clumps – grade 5 (iron overload); (H) Very large clumps and extra-cellular iron – Grade 6 (iron overload)*

2.3.2 Biomarkers of iron status and C-reactive protein

All venous blood samples for chemistry tests were collected in BD vacutainer® serum separator tubes, with gel (SST™, BD biosciences, Franklin Lakes, New Jersey, USA). The assays were performed using the automated Cobas® 6000 platform (Roche Diagnostics, Basel, Switzerland). Serum iron was measured using the IRON2 colorimetric assay, transferrin the TRSF2 immunoturbidometric assay, SF the Elecsys Ferritin electro-chemiluminescence immunoassay, and CRP the CRP4 particle-enhanced immunoturbidimetric assay.

2.3.3 Red cell indices and reticulocyte studies

All venous blood FBC samples were collected on the day of the procedure in 4 mL ethylenediamine tetra-acetic acid (EDTA) and stored at room temperature (18-26$^{\circ C}$) prior to analysis. Analysis was performed within 6 hours of collection on the automated XN-9000 instrument (Sysmex Corporation, Kobe, Japan). The haemoglobin, MCV, MCH, mean corpuscular haemoglobin concentration, red cell distribution width (RDW), reticulocyte production index and RET-H*e* results were obtained.

2.3.4 Supportive patient information

Clinical details regarding patient comorbidities, blood transfusion and medication were obtained from clinical files and the clinical REDCap® database. Laboratory results were extracted from the NHLS laboratory information system (LIS). The CD4 (Beckman Coulter Aquios CL) and HIV viral load (Roche COBAS AmpliPrep/TaqMan HIV-1 Test) were extracted for all patients with HIV. When relevant, the histological diagnosis in the database was confirmed by reviewing the report.

2.4 Data analysis:

Sample size was determined using BM ID as the primary outcome and SF as the primary predictor variable. Using a standard effect size of 0.58, a two-sided α of 0.05 and 80% power the required sample size of 96 was calculated. Data were analysed using STATA version 18.0 (Stata Corporation, College Station, Texas, USA). Categorical data were described using percentages and frequencies. Numerical data were described by medians and interquartile ranges, as data were non-parametrically distributed as determined by evaluation of histograms. Pearson's Chi-square or Fisher's exact tests were used to compare categorical data by BM iron status. Numerical data were compared using the Mann-Whitney U or Kruskal-Wallis tests.

Receiver operating characteristics (ROC) curve analysis was performed to assess the diagnostic accuracy of peripheral blood markers and indices for predicting BM iron status and to determine the optimal cut-off of each variable for distinguishing ID. The sensitivity and specificity information at each cut-off was interrogated manually and Youden's index was calculated to identify the optimal cut-off for each diagnostic test. Subgroup analysis was performed to assess differences between the male and

female study patients and those with and without inflammation as defined by CRP >5mg/L or ≤5mg/L, respectively. [39]

Univariable logistic regression was performed to identify parameters predictive of BM ID in the total population. A multivariable logistic regression analysis was subsequently conducted to evaluate the parameters as categorical variables using diagnostic cut-offs established based on the analysis of ROC-curves. This analysis was performed in the total population and the subgroup of those with inflammation as there were too few patients without inflammation for subgroup analysis. A combined proposed algorithm was produced using the variables predictive of BM ID on multivariable analysis. Sensitivity and specificity for this algorithm were calculated manually.

3. Results

In the first phase of the study, 116 patients were included; 97 iron replete and 19 with ID. Fifty-three did not meet inclusion criteria. In view of the small initial number of BM ID cases, a further 550 BM biopsies were reviewed retrospectively to enrich the study for ID. Of these, 525 were excluded and a further 23 patients with ID identified. After all exclusions, 139 patients were included for final analysis including 42 with ID (Figure 1).

The study population showed a high prevalence of anaemia (71%; n=99) irrespective of BM iron status, with low median haemoglobins in both the ID group and the iron replete group (table 1). The primary diagnosis was a malignancy in 81% (n=113), most commonly haematological, and 27% (n=35/129) were people living with HIV. Tuberculosis was a confirmed comorbidity in 5 patients, 3 of whom had HIV, but there were no cases of BM infiltration by tuberculosis. One hundred and thirty-four patients had CRP data of whom 72% (n=96/134) had a CRP >5mg/L indicating inflammation, which was associated with replete BM iron stores (p=0.029).

Table 1. Study population baseline characteristics stratified according to bone marrow iron stores.

Variables Total study population n = 139		Bone Marrow Iron Stores Deficient† n=42 (%)	 Replete n=97 (%)	P-value
Age (years)	Median (IQR)	54.5 (39.0-66.0)	50.6 (38.7-59.8)	0.265
Sex‡	Male	17 (40.5)	50 (51.6)	0.230
	Female	25 (59.5)	47 (48.5)	
Diagnosis	Malignancy§	34 (81.0)	79 (81.4)	
	Non-malignant	1 (2.4)	11 (11.3)	0.070
	Normal/non-diagnostic	7 (16.7)	7 (7.2)	
HIV status (n= 129)	Positive	7/38 (18.4)	28/91 (30.8)	0.150
CRP (n = 134)	Median (IQR)	9.0 (2.0-31.0)	24.0 (6.0-74.0)	0.029
	> 5mg/L (n=96)	23/37 (62.2)	73/97 (75.3)	0.133
Anaemia	Male (Hb < 13 g/dL)	12/17 (70.6)	35/50 (70.0)	0.963
	Female (Hb < 12 g/dL)	19/25 (76.0)	33/47 (70.2)	0.602
Haemoglobin	Median (IQR)	9.7 (8.3-12.2)	10.8 (7.9-12.7)	0.947
Iron studies				
- SF	Median (IQR)	48.5 (26-136)	413 (169-1072)	<0.001
	<30 µg/L	15 (35.7)	0 (0)	<0.001
	30 – 100 µg/L	14 (33.3)	12 (12.4)	
	101 – 300 µg/L	10 (23.8)	28 (28.9)	
	>300 µg/L	3 (7.1)	57 (58.8)	
- TSAT	Median (IQR)	10 (7-15)	21 (13-27)	<0.001
	<16%	32 (76.2)	33 (34.0)	<0.001
	<20%	34 (81.0)	44 (45.4)	<0.001
- SF and TSAT	30 – 100 & <16% (n=15)	10 (23.8)	5 (5.2)	0.002
	30 – 100 & <20% (n=17)	12 (28.6)	5 (5.2)	<0.001
	101 – 300 & <16% (n=18)	5 (11.9)	13 (13.4)	0.809
	101 – 300 & <20% (n=22)	5 (11.9)	17 (17.5)	0.404
- Tf/Log (SF)	>1.70	19 (45.2)	97 (100)	<0.001
MCV	Median (IQR)	82.3 (73.9-92.6)	89.5 (84.9-95.2)	<0.001
	≤80 fL	17 (40.5)	12 (12.4)	<0.001
MCH	Median (IQR)	26.3 (22.5-30.0)	29.2 (26.4-30.7)	0.001
	≤27 pg	23 (54.8)	27 (27.9)	0.002
RET-H*e*	Median (IQR)	28.9 (23.9-31.2)	32.3 (28.7-34.6)	<0.001
	<26 pg	16 (38.1)	11 (11.3)	<0.001
	26 – 31.5 pg	16 (38.1)	31 (32.0)	
	>31.5 pg	10 (23.8)	55 (56.7)	
BM sideroblasts	<10 %	40 (95.2)	75 (77.3)	0.030
	10 – 40 %	2 (4.8)	15 (15.5)	
	>40 %	0 (0)	7 (7.2)	

† Iron deficiency defined as BM iron stores grade 0 or 1. Case selection for iron deficient patients means prevalence cannot be inferred from this table.

‡ Biological sex

§ Diagnoses: lymphoma (n=56), plasma cell dyscrasia (n=28), myeloproliferative neoplasms (n=14), acute leukaemia (n=9), myelodysplastic neoplasms (n=3), chronic lymphocytic leukaemia (n=2), and non-haematological malignancy (n=1)

IQR – Interquartile range; HIV – Human immunodeficiency virus; SF – Serum ferritin; TSAT – Transferrin saturation; Tf – Transferrin; MCV – Mean cell volume; MCH – Mean cell haemoglobin; RET-He – Reticulocyte haemoglobin equivalent

Table 2. Optimal biomarker cut-offs for the diagnosis of bone marrow iron deficiency using CRP >5 mg/L as a marker of inflammation.

Parameter			Optimal cut-off	Sensitivity at cut-off	Specificity at cut-off	AUC^{ROC}	95% CI
SF	Total[†]	With ID[‡]	*(µg/L)*	(%)	(%)		
Total							
Both sexes[§]	134	37	80	69	94	0.88	0.82-0.95
Male	65	15	77	71	100	0.92	0.85-0.99
Female	69	22	57	64	94	0.85	0.75-0.95
CRP ≤5 mg/L							
Both sexes	38	14	74	93	96	0.97	0.92-1.00
Male	20	7	77	100	100	1.00	1.0-1.00
Female	18	7	68	86	91	0.94	0.82-1.00
CRP >5 mg/L							
Both sexes	96	23	193	78	75	0.84	0.74-0.94
Male	45	8	373	100	68	0.88	0.77-1.00
Female	51	15	146	80	75	0.80	0.83-1.00
TSAT			*(%)*				
Total	134	37	13.5	71	74	0.77	0.68-0.86
CRP ≤5 mg/L	38	14	14.0	86	92	0.89	0.76-1.00
CRP >5 mg/L	96	23	12.5	61	79	0.72	0.59-0.85
Tf/Log (SF)							
Total	134	37	1.40	62	94	0.83	0.75-0.91
CRP ≤5 mg/L	38	14	1.38	93	96	0.95	0.86-1.00
CRP >5 mg/L	96	23	0.86	78	68	0.77	0.65-0.89
RET-He			(pg)				
Total	134	37	31.4	76	58	0.70	0.60-0.80
CRP ≤ 5 mg/L	38	14	32.6	86	71	0.82	0.68-0.96
CRP > 5 mg/L	96	23	32.0	83	49	0.67	0.54-0.81

[†] Includes 134 patients with available CRPs including 37 of whom had ID
[‡] Iron deficiency defined as BM iron stores grade 0 or 1
[§] Biological sex
SF – Serum ferritin; TSAT – Transferrin saturation; Tf/log (SF) – Transferrin/Log(ferritin); RET-He – Reticulocyte haemoglobin equivalent; AUC^{ROC} – Area under the receiver operator characteristic curve; CI – Confidence interval

All patients with SF < 30 µg/L had BM ID, but this cut-off identified only 36% (n=15) of patients with ID. Many patients with ID had SF between 30 and 300 µg/L (Table 1). SF was the single best parameter for the diagnosis of ID with an area under the ROC curve (AUC^{ROC}) of >0.80 in all analyses (Table 2). An optimal SF cut-off of <80 µg/L for ID diagnosis was identified in the total group with sensitivity of 69% and specificity of 94% (Figure 3). Multivariable analysis confirmed the correlation of SF <80 µg/L with BM ID (Adjusted OR 23.5; 95% CI 4.3 – 129) (Table 3). Subgroup analysis of patients without inflammation identified a cut-off of 75 µg/L (n=38) with excellent sensitivity (93%) and specificity (96%) and a negative predictive value (96%) which is maintained at a SF cut-off of <80 µg/L (supplementary Table 2). In patients with inflammation a SF cut-off of <193 µg/L (n=96) maintained good sensitivity and specificity for ID (78% and 75%, respectively). A marginally higher SF cut-off of 200 µg /L had

essentially unchanged sensitivity (78%) and specificity (74%). Moreover, in patients with inflammation, a SF <193 µg/L was confirmed as a predictor of ID by multivariable analysis (Adjusted OR 10.5; CI 3.2 – 35).

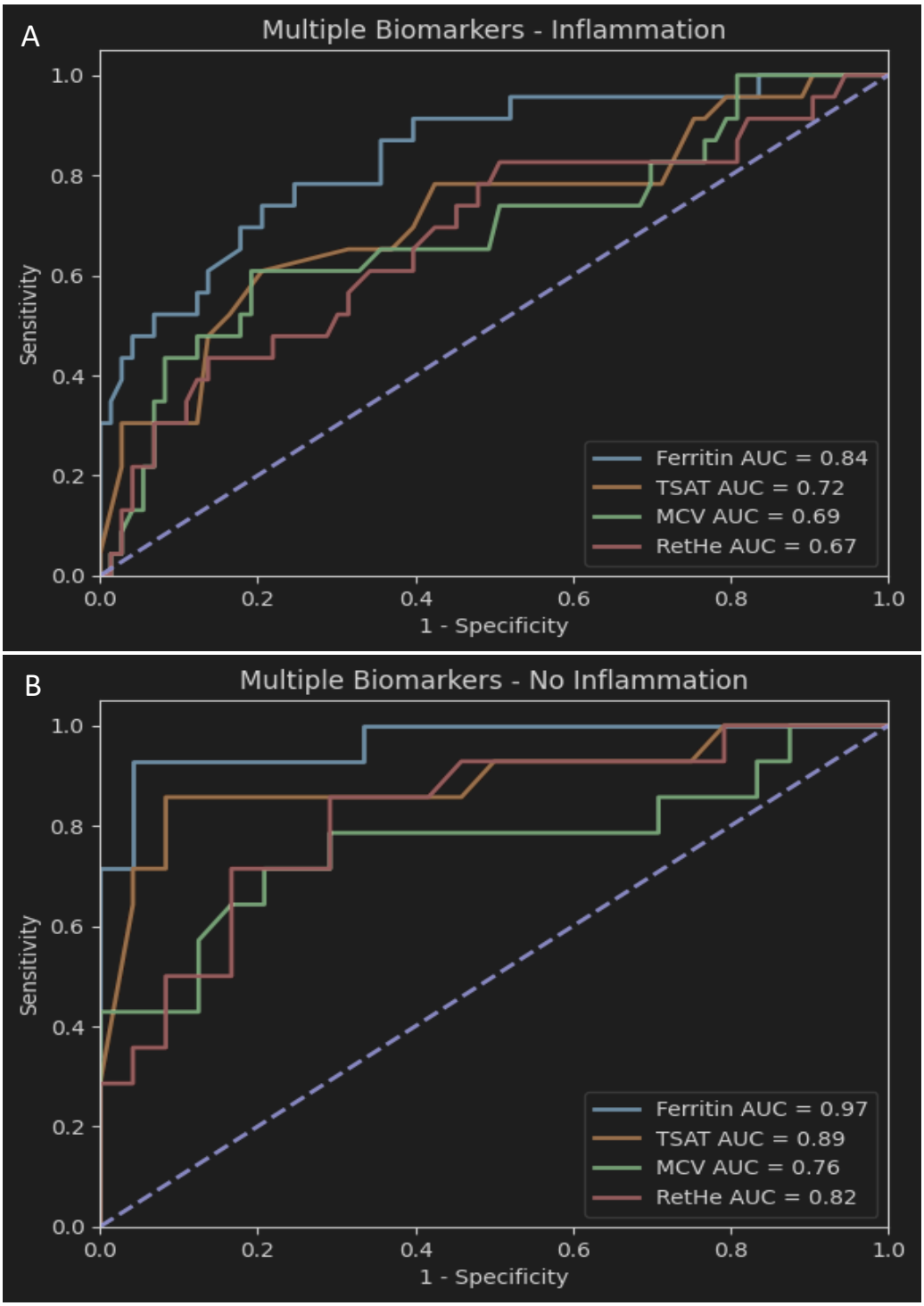

Figure 3: ROC curve analysis showing the performance of serum ferritin in predicting bone marrow iron deficiency.

(A) Biomarker performance in patients with CRP >5 mg/L; (B) Biomarker performance in patients with CRP ≤5 mg/L. ROC – Receiver operating characteristic; TSAT – Transferrin saturation; MCV - Mean cellular volume; RET-He – Reticulocyte haemoglobin equivalent; AUC – Area under the curve

ROC-curve analysis of patients with anaemia identified a SF cut-off of 199 µg/L (n=99) with sensitivity (81%) and specificity (84%) for the diagnosis of ID. In patients without anaemia 11 had ID and the diagnostic SF cut-off was 66 µg/L (n=40) with sensitivity (91%) and specificity (90%). However, Hb was included in the multivariable analysis and was not shown to be predictive of ID.

The median TSAT was 10% in patients with ID compared to 21% in those with iron replete stores (p<0.001). However, 45% (n=44) of patients with replete iron stores had TSAT <20%. Combining a SF of 30-100 µg/L with TSAT <20% was predictive of ID (p<0.001), identifying 12 of 14 patients with ID. No significant relationship could be demonstrated in patients with SF of 101-300 µg/L and TSAT <20% (p=0.4). The TSAT was confirmed to be a useful parameter for the diagnosis of ID in both the total population and subgroup analysis by CRP (all AUC^{ROC}s >0.7). The TSAT, when used as a categorical variable independent of SF, was shown to be predictive of ID by multivariable analysis in the total group and elevated CRP sub-group using cut-offs of <14% (Adjusted OR 3.8; 95% CI 1.1-12.8) and <13%, respectively (Adjusted OR 5.7; 95% CI 3.2-35.0). The TF/log SF ratio performed better than TSAT in all analyses, but with lower AUC^{ROC}s than SF and was excluded in multivariable analysis due to being highly correlated with SF.

Using SF <80 µg/L in those without inflammation and SF <200 µg/L and TSAT <13% in those with inflammation correctly classified 88% of all patients with high sensitivity (79%) and specificity (92%)

Table 3: Adjusted odds ratios for parameters predicting iron deficiency by multivariable logistic regression.

Population	Total	With ID	Adjusted OR	95% CI	P-value
Total cohort	134	37			
Ferritin ≤80 µg/L			23.5	4.3 – 129.0	<0.001
TSAT <14 %			3.8	1.1 – 12.8	0.033
CRP > 5 mg/L[†]	96	23			
Ferritin ≤193 µg/L			10.5	1.8 -18.1	0.003
TSAT <13%			5.7	3.2 – 35.0	<0.001

[†]Note that multivariable analysis could not be performed in patients with CRP ≤ 5 mg/L due to low numbers
TSAT – Transferrin saturation; CRP – C-reactive protein; OR – Odds ratio; CI – confidence interval

4. Discussion

This study, performed in a tertiary academic hospital, in an HIV-endemic region aimed to describe the relationship between deficient BM iron stores, peripheral blood biomarkers and CRP in an effort to optimise the diagnosis of ID. The study established new cut offs for the diagnosis of ID in patients with and without inflammation, indicated by a CRP >5 mg/L. Secondly, the TSAT was shown to be a valuable additional biomarker to improve specificity and the positive predictive value for ID diagnosis.

The SF was the best single biomarker for the diagnosis of BM ID despite the high rate of inflammation (71%) in our study population. The SF had a high AUC^{ROC}s on ROC-curve analysis, with a SF cut-off of 80 µg/L selected in the whole group regardless of inflammation. This SF cut-off is significantly higher than current SF laboratory thresholds used for ID diagnosis and interestingly had lower sensitivity (69%) than specificity (94%). Even in the absence of laboratory evidence of inflammation, the traditional SF cut-off of <15 µg/L, or even 30 µg/L, proposed by the World Health Organisation and other authors would have incorrectly classified many study patients with ID as being iron replete. [7,19,26,27,40] This would lead to missed diagnosis, undertreatment and/or unnecessary further investigation for causes of anaemia other than ID. A recent South African study of 57 HIV positive patients supports the use of a higher SF cut-off for ID diagnosis because 92% (n=12/13) of patients with BM ID had normal (>30 µg/L) or elevated SF levels. [36] Our evidence for a higher SF cut-off to diagnose ID in patients with inflammation or infection is similar to recommendations made by the World Health Organisation who recommend a SF <70 µg/L. [27] Similarly, a SF <100 µg/L for the diagnosis of ID in patients with chronic inflammation has been proposed by Cacoub, et al. [41]

In our study, a SF <80 µg/L in patients without inflammation had a high negative predictive value (96%) for ID. Therefore, a patient with a SF ≥80 µg/L can be regarded as iron replete with a high degree of confidence. In contrast, an elevated CRP >5 mg/L identified a subgroup in whom a higher SF cut-off of 200 µg/L should be used for ID diagnosis. This cut-off was slightly higher than the 193 µg/L selected by Youden's index and was chosen pragmatically for clinical ease of use and to favour slightly increased test sensitivity. [19] The cut-off of 200 µg/L is significantly higher than those previously suggested for ID diagnosis in patients with inflammation, but similar to those used in cardiac failure and inflammatory bowel disease. [7,23,24,42]

The high rate of inflammation in our study is expected given the high prevalence of malignancy (81%; n=113) and comorbidities, such as HIV infection (27%; n=35/129). [1,10–16] With the use of the 200 SF cut-off, many of these patients with inflammation could be diagnosed with ID. The identification and treatment of ID in these patients could lead to a significant improvement in their quality of life. Additionally, it allows for the exclusion of potentially significant and serious underlying causes of ID. [3] Furthermore, there are cost saving implications as patients with SF <80 µg/L would require no further testing to confirm the diagnosis. The similarity of the diagnostic SF cut-offs determined in sub-group analysis of patients with inflammation and of patients with anaemia is thought to be due to the correlation between anaemia and inflammation in our study population. This is an interesting finding that warrants further investigation in a study with a larger cohort of non-anaemic patients.

The Tf/log (SF) ratio, despite having excellent AUC^{ROC} did not offer any significant benefit to diagnosing ID in our study population. This is likely due to the outstanding performance of SF, therefore the addition of Tf to SF in this ratio weakened, rather than strengthened, its ability to predict ID. Transferrin saturation in contrast had slightly lower AUC^{ROC}s, but was identified by multivariable analysis as a good independent marker of ID using a cut-off of <14% (Adjusted OR 3.8; 95% CI 1.1 -12.8) in all patients and <13% (Adjusted OR 5.7; 95% CI 1.8 – 18.1) in those with inflammation. If the diagnosis of ID is based on either reduced TSAT or reduced SF there is a marked increase in test sensitivity, but with a loss of specificity that is not emphasised by those proposing this approach. [41,43] The use of both a reduced TSAT and a reduced SF, on the other hand, results in a loss of sensitivity but excellent test specificity. An in-depth understanding of this trade-off between test sensitivity and specificity is useful in planning low-cost diagnostic strategies. Based on our findings it is proposed that in a hospital setting with a high prevalence of inflammation SF, CRP and TSAT should be used in combination to diagnose ID as shown in figure 4. In our population, this combination improved the sensitivity from 69%, using SF <80 µg/L alone, to 79% without significantly worsening specificity (94% vs 92%).

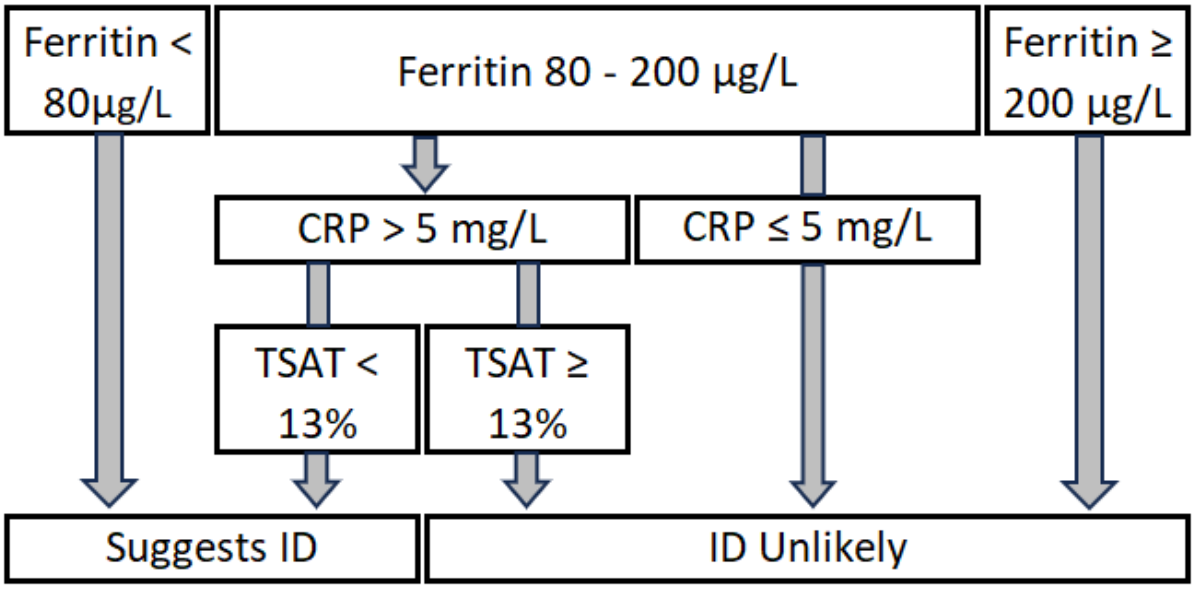

Figure 4. A proposed algorithm for the diagnosis of iron deficiency using CRP to stratify for inflammation.

CRP - C-reactive protein; TSAT - Transferrin saturation; ID - Iron deficiency

Lastly, red cell indices, MCV and MCH, were poorly predictive of ID at standard cut-offs identifying only 41% (n=17) and 55% (n=23) of patients with ID, respectively. ROC curve analysis confirmed cut-offs in keeping with the literature and common practice, but AUC^{ROC}s in the total group and in those with elevated CRP were poor. This supports the understanding that microcytosis and hypochromasia are less prominent in patients with coexistent ID and inflammation, and may be masked by comorbidities such as HIV, and drug therapies. [15,42] The reticulocyte parameter RET-H*e* was associated with ID, but

the cut-offs identified for the diagnosis of ID by ROC-curve analysis are higher than values seen in some other studies. A RET-H*e* <31 pg in the total group and 32 pg in those with elevated CRP had acceptable sensitivity (76% and 83%, respectively), but poor specificity (58% and 49%, respectively). [44,45] Interestingly the best AUC^{ROC} for RET-*He* was seen in patients without an elevated CRP, with a cut-off of < 32.6 pg (AUC^{ROC} 0.82), and a poor AUC^{ROC} (<0.7) was seen in patients with an elevated CRP. [42,43] Therefore, in contrast to findings from other studies, our data did not show any added value of RET-H*e* in identifying patients with ID in those with indeterminate SF results or concurrent inflammation, in this population. [7,46,47] The use of RET-H*e* in conjunction with the Tf/log (SF) as suggested by de Leur *et al.* in the alternative Thomas-plot had perfect specificity, but poor sensitivity limiting its use as a screening tool. [28] It is noted, however, that our study assessed the diagnosis of ID by deficient BM iron stores, not the role of RET-*He* in the management of ID. [29]

This single-centre study assessed patients referred to a tertiary haematology service in an HIV-endemic setting which may limit generalisability to other hospitalised patient populations. In addition, the high prevalence of certain conditions, such as lymphoma and myeloma, may have introduced bias. The use of CRP allows an objective assessment of inflammation, but the use of other markers such as the a1-acid glycoprotein or a panel of inflammatory biomarkers are under investigation and may provide a more comprehensive insight into the levels of inflammation.[22] Lastly, retrospective case-finding of ID cases, weakened the study design. The use of adequate BM samples as the diagnostic standard with a biomarker of inflammation gives the study strength and provides significant insight into the diagnosis of ID. Larger prospective studies which include BM iron studies and multiple biomarkers of inflammation are required to validate this diagnostic approach and the proposed cut-offs. These studies would ideally be performed in a population with a lower burden of HIV and malignancy to be more broadly applicable.

Conclusion

In this hospital-based haematology study population including a high proportion of patients with haematological malignancies, in an HIV-endemic region, the diagnostic gold-standard of BM iron stores was used to define ID. Serum ferritin levels and other laboratory biomarkers together with CRP were used to establish biomarker diagnostic thresholds. A serum ferritin of <80 µg/L reliably diagnosed ID in the absence of inflammation. In those with inflammation, ID could be diagnosed with high sensitivity (78%) and specificity (92%) if SF 80 – 200 µg/L was combined with TSAT of <13%.

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Declaration of interests:

None

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Appendices

Appendix 1: Supplementary tables

Supplementary Table 1: Univariable logistic regression for parameters predicting iron deficiency by multivariable logistic regression

Variables for analysis (n=139)		OR	95% CI	P-value
Age (Continuous variable)		1,01	0.99-1.04	0,292
Sex[‡] (Female)		1,56	0.75-3.26	0,232
HIV status (Positive)	(n=129)	0,51	0.20-1.29	0,155
CRP	(n=134)			
Continuous variable		0,99	0.99-1.00	0,119
≤5 mg/L		1,85	0.82-4.16	0,136
<10 mg/L		2,05	0.95-4.42	0,068
Transferrin saturation				
Continuous variable		0,91	0.87-0.96	<0.001
<14%		7,2	3.20-16.18	<0.001
<20%		5,12	2.15-12.19	<0.001
Ferritin				
Continuous variable		0,99	0.99-1.00	<0.001
<80.5 µg/L		33,8	11.80-97.05	<0.001
<100 µg/L		15,8	6.49-38.50	<0.001
Haemoglobin (continuous)				
Continuous variable		1,01	0.89-1.14	0,890
8.0-10.0 g/dL		4,11	1.38-12.23	0,011
>10 g/dL		1,33	0.50-3.56	0,566
MCV				
Continuous variable		0,93	0.89-0.97	<0.001
≤75 fL		8,25	2.71-25.09	<0.001
≤83.6 fL		5,7	2.55-12.70	<0.001
MCH				
Continuous variable		0,84	0.76-0.93	0,001
≤24 pg		6,18	2.37-16.14	<0.001
≤27.9 pg		3,49	1.64-7.45	0,001
RET-H*e*				
Continuous variable		0,86	0.79-0.93	<0.001
≤31 pg		4,37	1.93-9.89	<0.001
≤26 pg		4,81	1.99-11.65	<0.001
Sideroblasts				
Continuous variable		0,9	0.82-0.99	0,033
<20%		6,35	0.80-50.18	0,080

[†] Iron deficiency defined as BM iron stores grade 0 or 1. Case selection for iron deficient patients means prevalence cannot be inferred from this table

[‡] Biological sex

HIV – Human immunodeficiency virus; CRP – C-reactive protein; MCV – Mean cell volume; MCH – Mean cell haemoglobin; RET-He – Reticulocyte haemoglobin equivalent

Supplementary Table 2. Positive and negative predictive values at optimal biomarker cut-offs for the diagnosis of bone marrow iron deficiency

Parameter			Optimal cut-off	PPV at cut-off	NPV at cut-off
SF	Total[†]	With ID[‡]	*(µg/L)*	(%)	(%)
Total					
Both sexes[§]	134	37	80	81	89
Male	65	15	77	100	92
Female	69	22	57	83	85
CRP ≤5 mg/L					
Both sexes	38	14	74	93	96
Male	20	7	77	100	100
Female	18	7	68	86	91
CRP >5 mg/L					
Both sexes	96	23	193	50	92
Male	45	8	373	40	100
Female	51	15	146	57	90
TSAT			*(%)*		
Total	134	37	13.5	51	87
CRP ≤5 mg/L	38	14	14.0	86	92
CRP >5 mg/L	96	23	12.5	48	87
Tf/Log (SF)					
Total	134	37	1.40	80	87
CRP ≤5 mg/L	38	14	1.38	93	96
CRP >5 mg/L	96	23	0.86	43	91
RET-He			(pg)		
Total	134	37	31.4	41	86
CRP ≤ 5 mg/L	38	14	32.6	63	90
CRP > 5 mg/L	96	23	32.0	34	90

[†] Includes 134 patients with available CRPs including 37 of whom had ID
[‡] Iron deficiency defined as BM iron stores grade 0 or 1
[§] Biological sex
PPV – Positive predictive value; NPV – Negative predictive value; SF – Serum ferritin; TSAT – Transferrin saturation; Tf/log (SF) – Transferrin/Log(ferritin); RET-He – Reticulocyte haemoglobin equivalent; AUC^{ROC} – Area under the receiver operator characteristic curve; CI – Confidence interval

Appendix 2: REDCap® data capture instrument

Bone Marrow Iron Status

Record ID	_____
Folder Number	_____
Inpatient Status	◯ Inpatient ◯ Outpatient
Date of Birth	_____
Sex	☐ Male ☐ Female
Date of Procedure	_____
Primary Diagnosis	◯ Normal/ Non-diagnostic ◯ Lymphoma (incl not involved) ◯ AML ◯ ALL ◯ CLL ◯ MPN (incl CMML) ◯ MDS (excl CMML) ◯ Myeloma/ other plasma cell dyscrasia ◯ Non-haem malignancy ◯ Aplastic anaemia ◯ Pure red cell aplasia ◯ ITP ◯ Granulomatous inflammation/ TB ◯ Reactive / inflamation (incl HIV) ◯ Other
Histological Diagnosis	_____
Date of Test	_____
Co-morbidities	☐ Hypertension ☐ Diabetes ☐ Liver ☐ Renal ☐ Thyroid ☐ Cardiac ☐ Tuberculosis ☐ Unknown ☐ Other
Please specify	_____
Please specify "other"	_____

Medication	
Creatinine	
Date of Test	
C-Reactive Protein	
Serum Iron	
Transferrin	
% Saturation	
Ferritin	
HIV Status	☐ Positive ☐ Negative ☐ Unknown
Anti-Retroviral Therapy	☐ ABC ☐ 3TC/FTC ☐ EFV ☐ NVP ☐ TDF ☐ Dolu ☐ Lop/r ☐ Atz/r ☐ Bactrim ☐ Not documented ☐ Defaulted
Date of Test	
Viral Load	
Date of Test	
Haemoglobin	
MCV	
MCH	

MCHC	
RDW	
Plt	
Neut	
Lymph	
Mono	
Eos	
Baso	
Immature granulocyte	
Blasts	
Reticulocyte Production Index	
Reticulocyte Haemoglobin	
CD4 Count	
Date of Test	
Bone Marrow Iron Stores	◯ 0 ◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ Inadequate
Sideroblasts	◯ Observed ◯ Not Observed
% Non-pathological	

% Pathological	
% Ring	

Appendix 3: Ethics approval letter from the Faculty Research Ethics Committee

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

04 August 2021

HREC REF: 302/2021

A/Prof J Opie
Department of Pathology
NHLS-NGSH
Email: Jessica.opie@uct.ac.za
Student: david.richardson@nhls.ac.za

Dear A/Prof Opie

PROJECT TITLE: THE CORRELATION BETWEEN PERIPHERAL BLOOD IRON STUDIES, RETICULOCYTE INDICES AND BONE MARROW IRON STORES IN THE ASSESSMENT OF IRON DEFICIENCY-MMED CANDIDATE -DR DAVID RICHARDSON

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study, subject to adding the HREC contact details to the informed consent document.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020; 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 August 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr David Richardson will also be involved in this study.

Please quote the HREC REF 302/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF 302/2021sa

HUMAN RESEARCH ETHICS COMMITTEE
31 AUG 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.8.2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	31/8/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	07/08/2023		
HREC REF Number	302/2021	Current Ethics Approval was granted until	30 August 2023
Protocol title	The correlation between peripheral blood iron studies, reticulocyte indices and bone marrow iron stores in the assessment of iron deficiency.		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes	☐ No X	
If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	A/Professor Jessica Opie		
Department / Office Internal Mail Address	Division of Haematology, Room 6.06 Chris Barnard Bldg, Anzio Road, Observatory, 7925 (jessica.opie@uct.ac.za)		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ No X
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-submission@uct.ac.za)	☐ Yes	☐ No

If yes in 1.2 please complete section 1.3 below for invoicing purposes

1.3 Ethics Renewal Fee

Please **(tick ✓)**appropriate box for billing purposes:

Submission Type	*Description*	*New fee (Vat Incl.)*	*tick ✓*
Research funded solely from UCT departmental/ divisional/group budget	Annual evaluation of research progress report for re-certification	**R0,00**	x☐
Non-sponsored student research for degree purposes at UCT/Other Universities & Colleges	Annual evaluation of research progress report for re-certification	**R0,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R7000,00**	☐
Annual re-certification / Progress report (FHS016 Form)	Clinical Trial & International Grant Funded Research - Annual evaluation of research progress report for re-certification for Expedited review	**R3 710.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National grant funded research - Annual evaluation of research progress report for re-certification for Full Committee Approval	**R6000.00**	☐
Annual re-certification / Progress report (FHS016 Form)	National Grant funded research for Annual evaluation of research progress report for re-certification for Expedited review	**R1 500,00**	☐

NB: Protocols funded by UCT (e.g. departmental funding / student research) and by certain grant funding organizations (e.g. MRC, NRF, CANSA,) are exempt from these charges.

Please provide details for Invoicing, either complete section 1 or 2 :

1. Invoice billing – Directly to Sponsor

Sponsor's name	
Billing Address of Sponsor:	
Vat Number:	
Contact person	
Telephone number	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Email Address	
2. **Internal Journal Billing:**	
Fund Number:	
Cost Centre Number:	
Account Holder Name:	
Division of Account Holder:	

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open Enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐ X	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed ➔ Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	209
Number of participants enrolled, since last HREC Progress report (continuing review)	40
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	11

6. Cumulative summary of participants

Total number of participants who provided consent	209
Number of participants determined to be ineligible (i.e. after screening)	70

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Number of participants currently active on the study	N/A
Number of participants completed study (without events leading to withdrawal)	139
Number of participants withdrawn at participants' request (i.e. changed their mind)	0
Number of participants withdrawn by PI due to toxicity or adverse events	N/A
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	N/A
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	N/A
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	0

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
100 patients were initially enrolled in the study. Further patients were enrolled due to the high number of participants with inadequate bone marrow particles for assessment of iron stores. Repeat bone marrow iron stains and folder reviews have been performed for selected patients as per the protocol. The database has been completed and statistical analysis and write-up are under way. The report will be completed once secondary data analysis is available.

8. Protocol violations and exceptions (tick ✓ all that apply)

☐ X	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐ X	No Prior amendments have been made since the original approval

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
N/A

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☐ Not applicable X
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☐ Not applicable X

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☐ Not applicable X

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ No X
If yes, please explain:	

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
X ☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Insurance

Please confirm that valid no fault insurance is still in place? **(tick ✓)**			
☐ Yes	☐ No		☐ Not Applicable – N/A **X**
If yes, please complete the following:			
Insurer's name:			
Policy no.		*Coverage Period:	
For UCT sponsored studies please liaise the Insurance office via fhs.sponsorship@uct.ac.za regarding the required documentation and information required obtain a renewed UCT No-fault Insurance Certificate.			

14. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☐ No X
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

15. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	30 August 2023

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.10.2021
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	8/10/2020

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown

Comments to PI from the HREC	
Thank you for the deviation document	

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	05.10.2020		
HREC REF Number	R018/2017	Current Ethics Approval was granted until	30.05.2020
Protocol title	Bone marrow database 2005- current date		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ☐ No N/A		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	A/Prof Jessica Opie		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Department / Office Internal Mail Address	Division of Haematology jessica.opie@uct.ac.za

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐ No X
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		
1.3 Annual Approval for **full committee** review	- R 3450 (inclusive of vat)	
For invoicing purposes, please provide:		
Sponsor's name		
Contact person		
Address		
Telephone number		
Email Address		

2. List of documentation for approval

3. Protocol status (tick ✓)

☐	Open to enrolment
☐	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Number of participants enrolled to date	
Number of participants enrolled, since last HREC Progress report (continuing review)	
Additional number of participants still required	

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	

6. Cumulative summary of participants

Total number of participants who provided consent	
Number of participants determined to be ineligible (i.e. after screening)	
Number of participants currently active on the study	
Number of participants completed study (without events leading to withdrawal)	
Number of participants withdrawn at participants' request (i.e. changed their mind)	
Number of participants withdrawn by PI due to toxicity or adverse events	
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
The bone marrow database is an ongoing NHLS database of all BM biopsies performed from 2005 with results entered onto the laboratory information system. This data has been/will be used towards MMed projects in the department.

8. Protocol violations and exceptions (tick ✓ all that apply)

☐	No prior violations or exceptions have occurred since the original approval (5 months past expiry date)

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐X	No prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
☐ Yes	☐ No	☐ Not applicable
If yes, please describe:		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	☐ No	☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	☐ No	☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	☐ No
If yes, please explain:	

12. Level of risk (tick ✓)

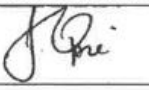
12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
☐	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	☐ No X
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

14. Signature

My signature certifies that the above is complete and correct.			
Signature of PI		Date	5 October 2020

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix 4: Research approval letter from Groote Schuur Hospital

Western Cape Government

GROOTE SCHUUR HOSPITAL

Enquiries: Mr Lionel Naidoo
E-mail: GSHResearch.Request@westerncape.gov.za

Professor J. Opie
Department of Pathology

E-mail: jessica.opie@uct.ac.za

Dear Professor Opie

RESEARCH PROJECT EXTENSION: The Correlation between Peripheral Blood Iron Studies, Reticulocyte Indices and Bone Marrow Iron Stores in the Assessment of Iron Deficiency – MMED Candidate Dr David Richardson

Your recent communication to the hospital refers.

The extension of your research is approved in accordance with **UCT Ethics** clearance, until **30 August 2024**

As previously mentioned,

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must always be maintained.
g) Once the research is complete, please submit a copy of the publication or report.
h) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.
i) All Clinical Trials to be registered on Clinicom with Michelle Riley.
michelle.riley@westerncape.gov.za

I would like to wish you every success with the project.

Yours sincerely

MR LIONEL NAIDOO
HEAD: ALLIED HEALTH
Date: 22 September 2023

C.C. Mr. L. Naidoo, Mr. A. Mohamed, Dr N. Khumalo, Professor V. Louw

G46 Management Suite, Old Main Building, Observatory 7925
Private Bag X, Observatory, 7935
Tel: +27 21 404 6288 fax: +27 21 404 6125
www.westerncape.gov.za/health

Appendix 5: Informed consent form

PARTICIPANT INFORMATION LEAFLET AND INFORMED CONSENT (July 2021)

STUDY TITLE: The correlation between peripheral blood iron studies, reticulocyte indices and bone marrow iron stores in the assessment of iron deficiency

Good day,

I am a team member from the Division of Haematology, researching the use of blood tests in predicting iron stores in the bone marrow.

Why is this study being done?
Iron deficiency is a common and treatable condition. Diagnosis is usually based on blood test which may be falsely normal in patients with certain medical conditions. This study will aim to improve the diagnosis of iron deficiency.

Why are you being asked to take part?
All participants, like you, referred by their doctor for bone marrow biopsy aged 18 years and over, are invited to participate in this study. This research study has been approved by the UCT Ethics in Research Committee.

If you are reading this, it means that you have already consented to have a bone marrow biopsy as part of clinical care to make a more accurate diagnosis regarding the cause of your illness.

Why would you want to participate?

- We will get information regarding your iron stores that will be available for your doctors, and they will be able to provide correct treatment, if necessary.

What will be required of you:

- We will draw approximately 10ml of peripheral blood from your arm vein. We will use your samples to perform a full blood count studies, iron studies and a marker of inflammation (CRP).
- We need permission to access your medical information and blood results.

What information will be collected and how it will be stored:
The following information will be collected from the medical folder:

- Date of birth, sex, HIV status (including viral load and CD4 count), full blood count, reticulocyte studies, CRP, kidney function, your diagnosis and reason for the bone marrow biopsy.
- This information will be stored in a secure online research database.

Access to this information will only be available to the study co-ordinators.
The information will be separated from your name and other personal information.

Only the study investigators will have access to both your personal information and the results of our tests.

Who are the team members that will be helping with collecting samples for the research?
All doctors and nurses working in E5 Clinical Haematology department will be helping with collection samples.

Please note:

- All the information that we get about you will be kept strictly confidential
- You may choose to stop participating in the study at any point without any consequences.
- You may have the bone marrow without being part of the study
- There is no money offered for your participation
- The results of the study are expected to be completed in 2022

Thank you for considering being part of this important study.

If you have any questions about the study, please contact:

Dr David Richardson
david.richardson@nhls.ac.za
021 404 4018
0845331278

I _____ (Full name of participant), hereby consent to participate in this study.

Participant/Guardian _____ Date: _____
Signature_____

Witness (if the participant is not literate)

_____ Date: _____ Signature_____